Received: 12 October 2022 | Revised: 13 January 2023 | Accepted: 20 February 2023

DOI: 10.1002/psp4.12949

ARTICLE

PK/PD modeling and simulation of the in vitro activity of the combinations of isavuconazole with echinocandins against *Candida auris*

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Abstract

In vitro combination of echinocandins and isavuconazole against the emerging species *Candida auris* is mainly synergistic. However, this combination has not been evaluated in clinical settings. A pharmacokinetic/pharmacodynamic modeling and simulation approach based on in vitro data may be helpful to further study the therapeutic potential of these combinations. Therefore, the aims of this study were to characterize the time course of growth and killing of *C. auris* in response to the combination of the three approved echinocandins and isavuconazole using a semimechanistic model and to perform model-based simulations in order to predict the in vivo response to combination therapy. In vitro static time-kill curve data for isavuconazole and echinocandins combinations against six blood isolates of *C. auris* were best modeled considering the total killing of the fungal population as dependent on the additive effects of both drugs. Once assessed, the predictive performance of the model using simulations of different dosing and fungal susceptibility scenarios were conducted. Model-based simulations revealed that none of the combinations at standard or higher dosages would be effective against the studied isolates of *C. auris* and it was predicted that the combinations of isavuconazole with anidulafungin or caspofungin would be effective for minimum inhibitory concentrations up to 0.03 and 0.06 mg/L respectively, whereas the combination with micafungin would lead to treatment failure. The current approach highlights the importance of bridging the in vitro results to the clinic.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

In previous in vitro studies, synergism was demonstrated for the combination of isavuconazole with echinocandins against *Candida auris*.

WHAT QUESTION DID THIS STUDY ADDRESS?

How can the in vitro information of the efficacy of anti-*Candida* drug combinations be described by a semimechanistic pharmacokinetic/pharmacodynamic (PK/PD) model.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This is the first study in which in vitro data based PK/PD modeling and simulation has been applied for antifungal combinations. The model was able to characterize properly the antifungal activity of isavuconazole in combination with echinocandins against the emerging and multiresistant species *Candida auris*. Synergism was found in vitro for the combination of isavuconazole with echinocandins. Model-based simulations revealed that none of the combinations at standard or higher dosages would be effective against the studied isolates.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The current approach highlights the importance of bridging the in vitro results to the clinic. By linking the in vitro based PK/PD model to population PK clinical information, combined antifungal therapy was translated into a clinical setting.

INTRODUCTION

Invasive candidiasis is the most common fungal infection and *Candida* is the third or fourth most common cause of nosocomial infection in patients in the intensive care unit, only surpassed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Candida albicans* is the main etiological agent, but, in the last decades, there has been an epidemiological drift and the incidence of non-*C. albicans* invasive candidiasis has grown, accounting for half of the cases worldwide.[1] In the last decade, a new species has emerged and has become a serious threat to healthcare systems: *Candida auris*. *C. auris* was first described in Japan in 2009, and, since then, it has caused notable outbreaks in countries, such as Spain, India, or the United States. Its high persistence in the hospital environment, difficulties with proper identification, and multidrug resistance make *C. auris* a challenging pathogen to control and treat. Organizations, including the United States Center for Disease Control and Prevention and the World Health Organization, have classified *C. auris* as an "urgent threat" and a pathogen of global health interest.[2,3]

Echinocandins are the recommended first-line treatment to cope with *C. auris* infections.[4] However, resistance to these drugs along with therapeutic failures has been reported.[5] Because of the current shortage of therapeutic options and the risk of treatment failure, combination therapies, as alternative strategy, are being further investigated. Recent studies have evaluated the interactions of antifungal drugs against *C. auris*,[6–8] or the combination of antifungal drugs with other antimicrobial agents.[9–11] We recently studied the in vitro time-kill activity of the combinations of echinocandins and isavuconazole, the newest and safer addition to the triazole antifungal group against *C. auris* and concluded synergy and fungistatic activity, in contrast to the reduced activity of monotherapies.[12] Additionally, the synergy observed in vitro against *C. auris* with the combination of isavuconazole and echinocandins in other studies[13,14] and also with isavuconazole and micafungin against other species of *Candida*,[15] supports the interest of further characterizing this interaction in a strictly quantitative fashion to ultimately support optimal treatment and dosing regimen selection.

However, in vitro synergism may not correlate with a successful clinical outcome.[16] pharmacokinetic/pharmacodynamic (PK/PD) modeling and simulation of antimicrobial in vitro data is a tool that can bridge in vitro results to in vivo scenarios and, thus, it may guide in the design of further studies and therapeutic decision making.[17]

Therefore, the aims of this study were to characterize the time course of growth and killing of *C. auris* in response to the combination of the three approved echinocandins and isavuconazole using a mathematical model and to perform model-based simulations in order to predict the antifungal response to combination therapy.

MATERIALS AND METHODS

Fungal strains and time-kill kinetics

The dataset used for the mathematical model building was obtained from a previously reported static time-kill curve study[12] for isavuconazole and echinocandins

combinations against six clinical blood isolates of *C. auris* (CJ94, CJ97, CJ98, CJ99, CJ100, and CJ102) from an outbreak in the Hospital Universitario y Politécnico La Fe (Valencia, Spain).[18] The minimum inhibitory concentrations (MICs) for isavuconazole, anidulafungin, caspofungin, and micafungin were 0.06, 0.125, 0.25, and 0.125 mg/L, respectively. Therefore, all isolates were classified as wild-type.[19]

Static time-kill curve experiments were carried out on flat-bottom microtiter plates in Roswell Park Memorial Institute (RPMI) medium, with a final volume per well of 200 µL at 37°C for 48 h. The *C. auris* blood isolates were grown at 37°C for 24 h prior to the start of each experiment to obtain fungal cultures in early logarithmic phase growth. Cells were then suspended in RPMI medium to achieve a starting inoculum size of $1–5\times10^6$ CFU/mL and added to the microtiter plate containing different concentrations of the antifungal agent ending with a starting inoculum size of $1–5\times10^5$ CFU/mL. The selection of drug concentrations in combination was based on previous checkerboard assay results.[12] These concentrations were 0.125, 0.25, 2, and 4 mg/L for isavuconazole and ranged from 0.5 to 4 mg/L for anidulafungin, caspofungin, or micafungin. Growth control was also determined by adding the inoculum to wells containing RPMI medium without drugs. To assess the interaction between drugs correctly, the concentrations assayed in the combinations were also studied in monotherapy simultaneously. Samples for viable counts were taken at 0, 2, 4, 6, 8, 24, and 48 h, plated in triplicate onto Sabouraud dextrose agar, and incubated for 24–48 h at 37°C. Assays were conducted in independent duplicate experiments. The lower limit of detection was 5 CFU/mL.

In vitro semimechanistic pharmacodynamic model

As a previous step to the modeling of combination therapy, each drug in monotherapy was modeled first, obtaining information regarding the best structural model and initial parameter estimates. A single-population structural model defined by the following equation best captured the activity of isavuconazole and the echinocandins alone:

$$\frac{dN}{dT} = \left[k_{growth} \times \left(1 - \frac{N}{N_{max}}\right) \times \left(1 - e^{-\alpha t}\right) - \frac{E_{max} \times C^h}{EC_{50}^h + C^h}\right] \times N \quad (1)$$

where dN/dt is the change in the number of *Candida* cells as a function of time, k_{growth} is the growth rate constant (h^{-1}) of *Candida*, N is the number of viable cells (log CFU/mL), N_{max} is the maximum total density of fungal population experimentally determined (log CFU/mL), and α accounts for the delay in growth observed due to experimental settings. E_{max} is the maximum effect produced by the drug (h^{-1}), C is drug concentration at time t (mg/L), EC_{50} is the concentration of the drug necessary to achieve half the maximum effect (mg/L), and h is the Hill factor, which modifies the steepness of the slope and smoothens the concentration-effect curve.

The best structural model that fitted all isavuconazole plus echinocandin combinations was defined by the following equation:

$$\frac{dN}{dT} = \left[k_{growth} \times \left(1 - \frac{N}{N_{max}}\right) \times \left(1 - e^{-\alpha t}\right) - \text{"combined effect"}\right] \times N \quad (2)$$

where the "combined effect" was formulated as follows, describing the total killing of the fungal population as dependent on the additive effects of both drugs. Additionally, the interaction was evaluated using an empirical interaction function to test for statistically significant differences from additivity:

$$\text{Combined effect} = EFF_{ISV} \times \left(1 + \frac{EFF_{CANDIN}}{EFF_{CANDIN} + EFF_{ISV}}\right)^{Int} + EFF_{CANDIN} \times \left(1 + \frac{EFF_{ISV}}{EFF_{ISV} + EFF_{CANDIN}}\right)^{Int} \quad (3)$$

EFF_{ISV} and EFF_{CANDIN} are the effect exerted by isavuconazole and the echinocandins, respectively, defined as an E_{max} sigmoidal effect and Int is the parameter that reflects the drug–drug interaction. A positive value of Int reflects synergism and a negative value defines indifference or antagonism.[20]

Data analysis

Log CFU/mL data from time-kill studies were analyzed with NONMEM version 7.4.3 (ICON plc), with first order conditional estimation (FOCE) as the estimation method, and an additive error model. As six clinical isolates were analyzed, interindividual variability (IIV) was investigated. Additionally, interoccasion variability (IOV) was also explored to account for potential differences between experiments or sample preparation. Model performance was evaluated based on precision of parameter estimates, changes in objective function value, and visual inspection of goodness-of-fit. Final model selection was also assessed by visual predictive check plots (VPCs) and nonparametric

bootstrap analysis ($n = 1000$). Perl-speaks-NONMEM (PsN) was used as run manager and Pirana as workbench.

PK/PD simulations

The developed semimechanistic model was used to perform simulations of different dosing and fungal susceptibility scenarios. For this purpose, human PK population models were extracted from literature for isavuconazole,[21] anidulafungin,[22] caspofungin,[23] and micafungin,[24] and linked to the PK/PD model. A summary description of the PK parameters and covariates used for simulations of PK profiles are shown in Table S1.

PK/PD simulations were conducted sequentially. First, the PK profiles of 1000 virtual patients were simulated and the total plasma concentrations were corrected for the free, unbound drug, considering the protein binding reported in literature for isavuconazole (98%), anidulafungin (99%), caspofungin (95%), and micafungin (99.9%).[25] Next, databases were created with the typical free concentrations to serve as the input for the PK part of the developed PK/PD models. Finally, the time courses of log CFU/mL after 1-week of treatment for 1000 individuals were predicted by applying the final PK/PD models.

The first scenario tested aimed to explore and compare the drug efficacy, expressed as either fungal burden reduction or suppression of growth, after different dosing schedules. Licensed standard dosages and alternative dosing regimens were simulated. Licensed regimens included: isavuconazole, 200 mg/8 h first 48 h, and 200 mg/day from day 3 onward; anidulafungin, 200 mg loading dose and 100 mg/day; caspofungin, 70 mg on day 1 followed by 50 mg/day from day 2; and micafungin, 100 mg/day. Alternative regimens included: isavuconazole, 400 mg/8 h first 48 h, and 200 mg/day after; anidulafungin, 200 mg loading dose and 200 mg/day; caspofungin, 100 mg/day; and micafungin, 600 mg/day. Alternative doses were based on proposals from other reports and/or clinical guides.[22–24,26] For the combination therapy of isavuconazole and echinocandins, four different dosing regimens were tested: (i) standard treatment of both isavuconazole and the echinocandin; (ii) standard dosing schedule of isavuconazole plus alternative treatment of echinocandin; (iii) alternative treatment of isavuconazole plus standard dosing of echinocandin; And (iv) alternative treatment of both isavuconazole and echinocandin.

Moreover, because all isolates in the present study shared the same MIC for each drug studied, different MIC scenarios were tested following an equation that relates the EC_{50} of a drug with the MIC[27]:

$$\mathrm{MIC} = \left(\frac{d}{E_{\max} - d}\right)^{1/h} \times \mathrm{EC}_{50} \quad (4)$$

where d is a drug-independent constant and h is the Hill factor. The EC_{50} value for each MIC scenario was then included in the PK/PD model and simulations were performed similarly.

RESULTS

PK/PD model and model evaluation

Final model parameters and the standard error of the estimates for combination therapies, alongside bootstrap estimations for every combination are presented in Table 1. Model parameters in the three combinations were estimated with RSE less than 20%, which alongside the 95% confidence interval (CI) obtained by bootstrapping, indicated model stability and a proper estimation of parameters. The EC_{50} of isavuconazole was similar in the three combinations (0.0683, 0.0554, and 0.0584 mg/L for the combinations of isavuconazole with anidulafungin, caspofungin, and micafungin, respectively). The EC_{50} of anidulafungin and micafungin were also similar (0.176 and 0.171 mg/L) to one another, whereas caspofungin's EC_{50} was almost three times higher (0.452 mg/L). Additionally, the EC_{50} of isavuconazole decreased remarkably in combinations compared to the monotherapies. The estimates of EC_{50} (RSE expressed as coefficient of variation) for the drugs in monotherapy were 0.364 mg/L (14%) for isavuconazole, 0.435 mg/L (20%) for anidulafungin, 0.221 mg/L (4%) for caspofungin, and 0.242 mg/L (35%) for micafungin. The interaction parameter Int was positive in every combination, which, alongside a 95% CI not overlapping zero, allowed to classify the drug interactions as synergistic. Additionally, similar to the analysis of single-agent activity, neither the inclusion of IIV nor IOV improved the model fit, hence, those variabilities were absent from the final model. Thus, variability was solely defined by the residual model, which was additive. Goodness-of-fit plots and VPCs that show adequate model fit are provided in Figures 1–3.

Simulations

Total and unbound concentration-time profiles of each drug after standard and alternative intravenous infusion dosing regimens were simulated for 1000 virtual patients over a week (Figures S1 and S2). As depicted in Figure 4, none of the simulated dosing scenarios for any combination showed successful activity against the studied *C. auris* isolates, as the simulated responses did not result in a decrease in fungal burden.

Additional simulations were performed over a 1-week period for various MIC scenarios ranging from 0.015 to

TABLE 1 Parameter estimates of the final PD model for isavuconazole plus echinocandin combinations.

		Estimate [RSE (95% CI)]		
Parameter	**Description**	**ISV + ANF**	**ISV + CSP**	**ISV + MCF**
k_{growth} (h^{-1})	Fungal growth rate constant	0.158 [fixed]	0.140 [fixed]	0.145 [fixed]
E_{maxISV} (h^{-1})	Maximum kill rate constant of isavuconazole	0.0198 [0% (0.0182–0.0210)]	0.0168 [3% (0.0160–0.0177)]	0.0176 [3% (0.0164–0.0186)]
EC_{50ISV} (mg/L)	Concentration of isavuconazole at which 50% of the E_{maxISV} is achieved	0.0683 [5% (0.0580–0.0799)]	0.0554 [9% (0.0469–0.0658)]	0.0511 [3% (0.0476–0.0543)]
h_{ISV}	Hill factor for isavuconazole	1.58 [3% (1–15-2.05)]	1.16 [11% (0.94–1.41)]	1.12 [6% (0.95–1.33)]
$E_{maxCANDIN}$ (h^{-1})	Maximum kill rate constant of the echinocandin	0.0272 [3% (0.0250–0.0290)]	0.0157 [6% (0.0137–0.0174)]	0.025 [4% (0.023–0.027)]
$EC_{50CANDIN}$ (mg/L)	Concentration of echinocandin at which 50% of the $E_{maxCANDIN}$ is achieved	0.176 [9% (0.148–0.215)]	0.452 [9% (0.376–0.534)]	0.171 [9% (0.142–0.199)]
h_{CANDIN}	Hill factor for the echinocandin	1 [fixed]	1.37 [8% (1.23–1.63)]	1 [fixed]
α	Delay in fungal growth	0.162 [4% (0.152–0.174)]	0.161 [4% (0.148–0.178)]	0.158 [4% (0.145–0.171)]
N_{max} (log CFU/mL)	Maximum fungal density	8 [fixed]	8 [fixed]	8 [fixed]
Int	Interaction parameter	0.55 [13% (0.42–0.67)]	1.14 [10% (0.93–1.39)]	0.41 [18% (0.28–0.56)]
σ (log CFU/mL)	Additive residual error	0.30 [2% (0.29–0.31)]	0.28 [3% (0.27–0.29)]	0.27 [6% (0.26–0.28)]

Abbreviations: 95% CI, 95% confidence interval obtained from a nonparametric bootstrap ($n = 1000$). ISV + ANF, isavuconazole plus anidulafungin; ISV + CSP, isavuconazole plus caspofungin; ISV + MCF, isavuconazole plus micafungin; PD, pharmacodynamic; RSE, relative standard error expressed as coefficient of variation.

0.06 mg/L for isavuconazole, from 0.015 to 0.125 mg/L for anidulafungin and micafungin, and from 0.015 to 0.25 mg/L for caspofungin. The simulation outcomes revealed that combinations of isavuconazole with anidulafungin or caspofungin were able to inhibit fungal growth in the first 24 h and stop fungal growth from 24 h onward. There were no differences in treatment outcomes between men and women. Conversely, the combination of isavuconazole and micafungin was not successful for the evaluated doses and MIC scenarios. The combined dosing schedules and MIC scenarios for which fungal growth was inhibited are provided in Table 2. The drug combination and doses that would lead to higher antifungal coverage (all six MIC scenarios) was the use of alternative dosages of both isavuconazole (400 mg every 8 h, first 48 h, followed by 200 mg daily) plus caspofungin (100 mg daily; Figure 5).

As expected, all alternative doses in drug combinations attained a higher antifungal coverage compared to the labeled standard combination dosing schedules. In fact, combinations with currently used standard doses of isavuconazole and anidulafungin would only inhibit fungal growth if MIC less than or equal to 0.015 mg/L for both drugs. In the case of the combination with caspofungin, standard doses would only inhibit fungal growth if MIC less than or equal to 0.015 mg/L for isavuconazole and MIC less than or equal to 0.03 mg/L for caspofungin.

DISCUSSION

In contrast to the lack of PK/PD modeling studies for antifungal combinations, experience with antibacterial combinations is more extensive.[28–30] To our knowledge, this is the first study in which in vitro data based PK/PD modeling and simulation has been applied for antifungal combinations.

Because infections caused by resistant or monotherapy poor-responding *Candida* were not frequent until the emergence of *C. auris*, there is little clinical evidence regarding combination therapy. Consequently, there are no official recommendations for optimal combination therapy beyond the amphotericin B plus flucytosine combination for some specific cases.[26]

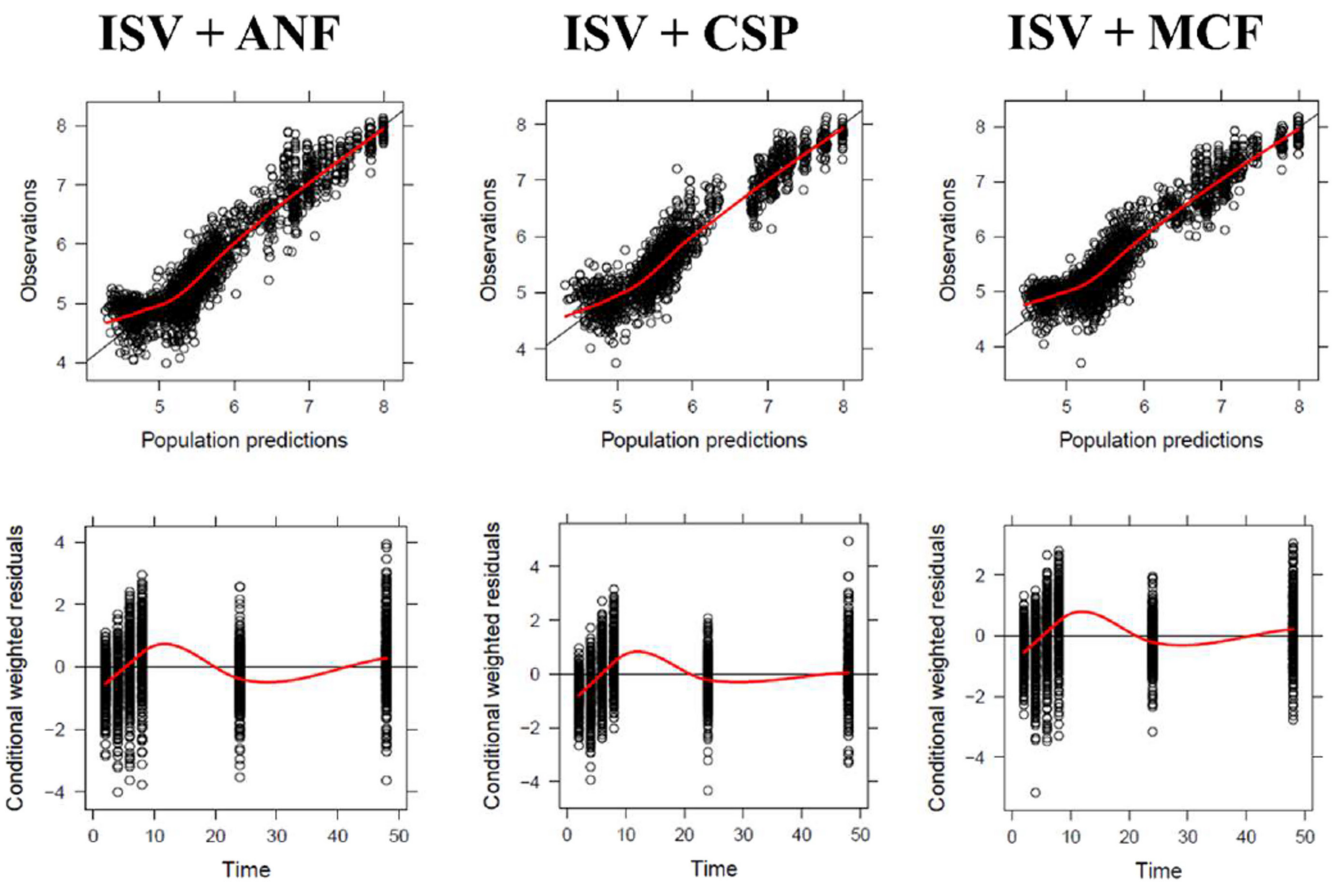

FIGURE 1 Observed fungal counts (log CFU/mL) versus population predictions (top row) and conditional weighted residuals (CWRES) over time (bottom row) plots for isavuconazole plus anidulafungin (ISV + ANF), isavuconazole plus caspofungin (ISV + CSP) and isavuconazole plus micafungin (ISV + MCF). The red lines are smooth lines showing the trend in the observations.

PK/PD modeling approaches have shown to be a useful tool to explore antimicrobial combination therapies.[20,31,32] In the current study, synergism was found in vitro for the combination of isavuconazole with echinocandin. The antifungal activity of isavuconazole combined with echinocandins in the present study was successfully characterized by a sigmoidal E_{max} model which included a previously described empirical interaction function for antibacterial combinations.[20] The model fit the data reasonably well. Although there was a slight underprediction of the effect of high-dose combinations at 48 h, given the little antifungal effect and the PK properties of the drugs, it did not affect the simulations and the conclusions driven from them. The interaction parameter Int obtained for each isavuconazole-echinocandin combination supported synergistic interactions, in agreement with the conclusions of the checkerboard assays and analysis with different approaches: the fractional inhibitory concentration index, Greco universal response surface approach, and Bliss interaction model.[12] The EC_{50} and E_{max} estimated for isavuconazole were similar in all combinations, indicating that the effects of each echinocandin on the PDs of isavuconazole were equivalent. Furthermore, there was a remarkable six-fold decrease on the EC_{50} of isavuconazole when combined with echinocandins. This aligned with the main hypothesis explaining the mechanistical basis for azole-echinocandin synergism. Echinocandins disrupt cell wall synthesis by inhibiting 1,3-β-D-glucan synthase, which, apart from the antifungal activity caused by the disruption itself, could also help to enhance the effect of the azole by increasing the access to the cell, where these drugs inhibit the biosynthesis of ergosterol.[15,33] Additionally, the EC_{50} of anidulafungin and micafungin were also lower compared to monotherapy and were about the same for both drugs, whereas the EC_{50} of caspofungin in combination was almost three times higher than those of anidulafungin and micafungin, supporting the lower potency identified by time-kill curves.

In our study, the alternative dosages used for simulations were based on proposed dosing regimen from the literature, where the authors concluded based on Monte Carlo simulations that higher echinocandin doses would be needed if the MICs of anidulafungin and caspofungin exceed 0.06 mg/L and that for micafungin exceeds 0.03 mg/L.[23,34,35] We also considered the recommended high dosing for echinocandins[26] and feedback provided

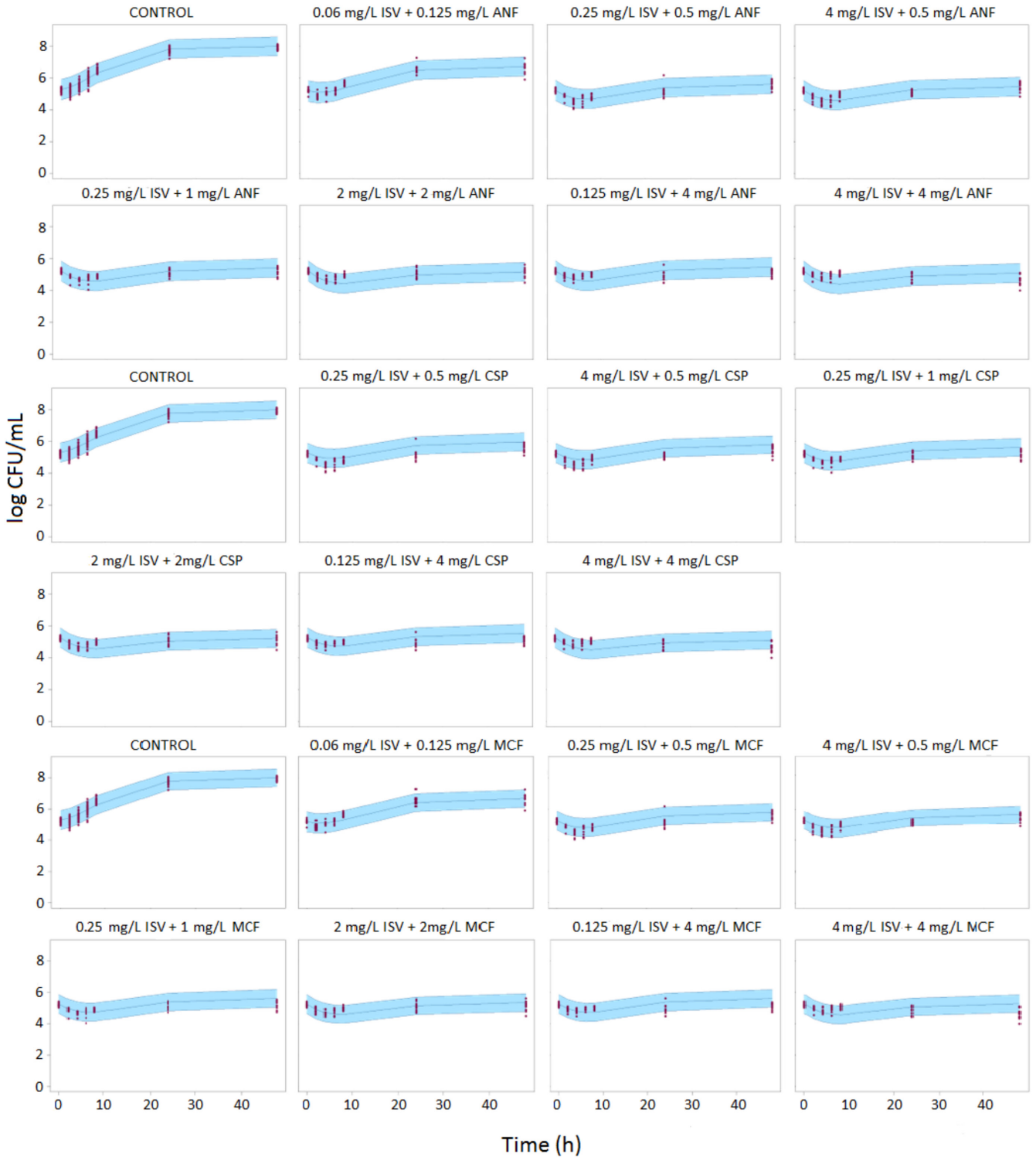

FIGURE 2 VPCs for the final model of isavuconazole plus anidulafungin/caspofungin/micafungin with the observed fungal counts (full circles), the mean prediction (solid line) and 95% prediction interval (shaded area) of the simulations. ANF, anidulafungin; CFU, colony forming units; CSP, caspofungin; ISV, isavuconazole; MCF, micafungin; VPCs, visual predictive check.

by the attending physicians. No therapeutic window has been established yet for isavuconazole, although a recent study identified 4.87 and 5.13 mg/L in serum to be the thresholds for dose-limiting toxicity.[36] Simulated mean concentrations in our study were below those values. Some individuals exceeded these thresholds but showed only gastrointestinal and no serious adverse events.

In contrast to the synergism detected in vitro for the combination of isavuconazole with echinocandin with different analysis,[12] when PK/PD simulations were

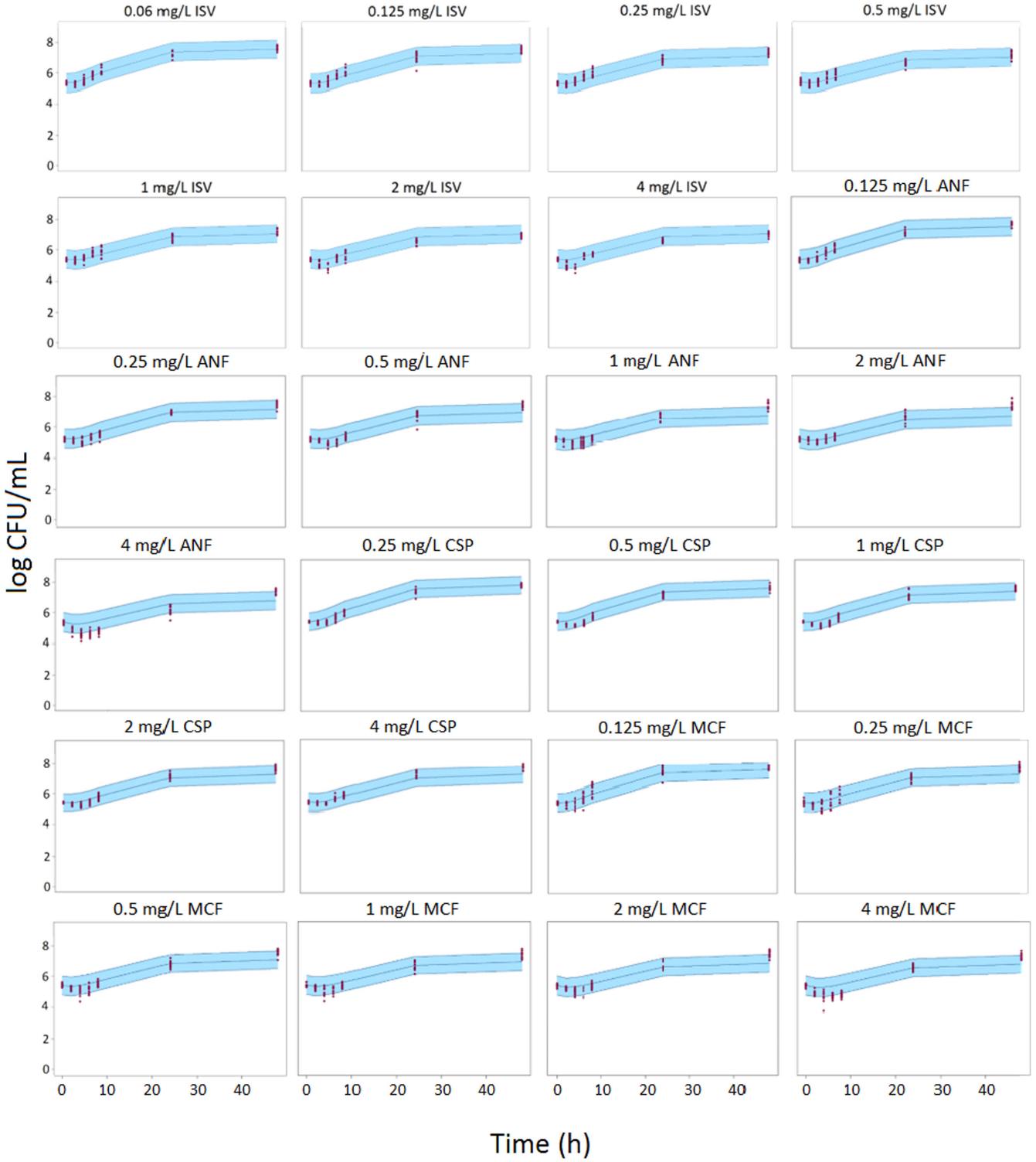

FIGURE 3 VPCs of the final combination models for each drug alone, with the observed fungal counts (full circles), the mean prediction (central solid line) and 95% model prediction interval (shaded area) of the simulations. ANF, anidulafungin; CSP, caspofungin; ISV, isavuconazole; MCF, micafungin; VPCs, visual predictive check.

conducted to generate expected kill curves for virtual patients, it was revealed that none of the combinations at standard or higher dosages would be effective.

Simulations for lower MICs showed that the combination of isavuconazole and micafungin was not successful for the evaluated doses and MIC scenarios. Conversely, combinations of isavuconazole with anidulafungin or caspofungin were able to inhibit fungal growth, depending on the dosing regimens tested for MICs up to 0.03 mg/L for isavuconazole and 0.06 mg/L

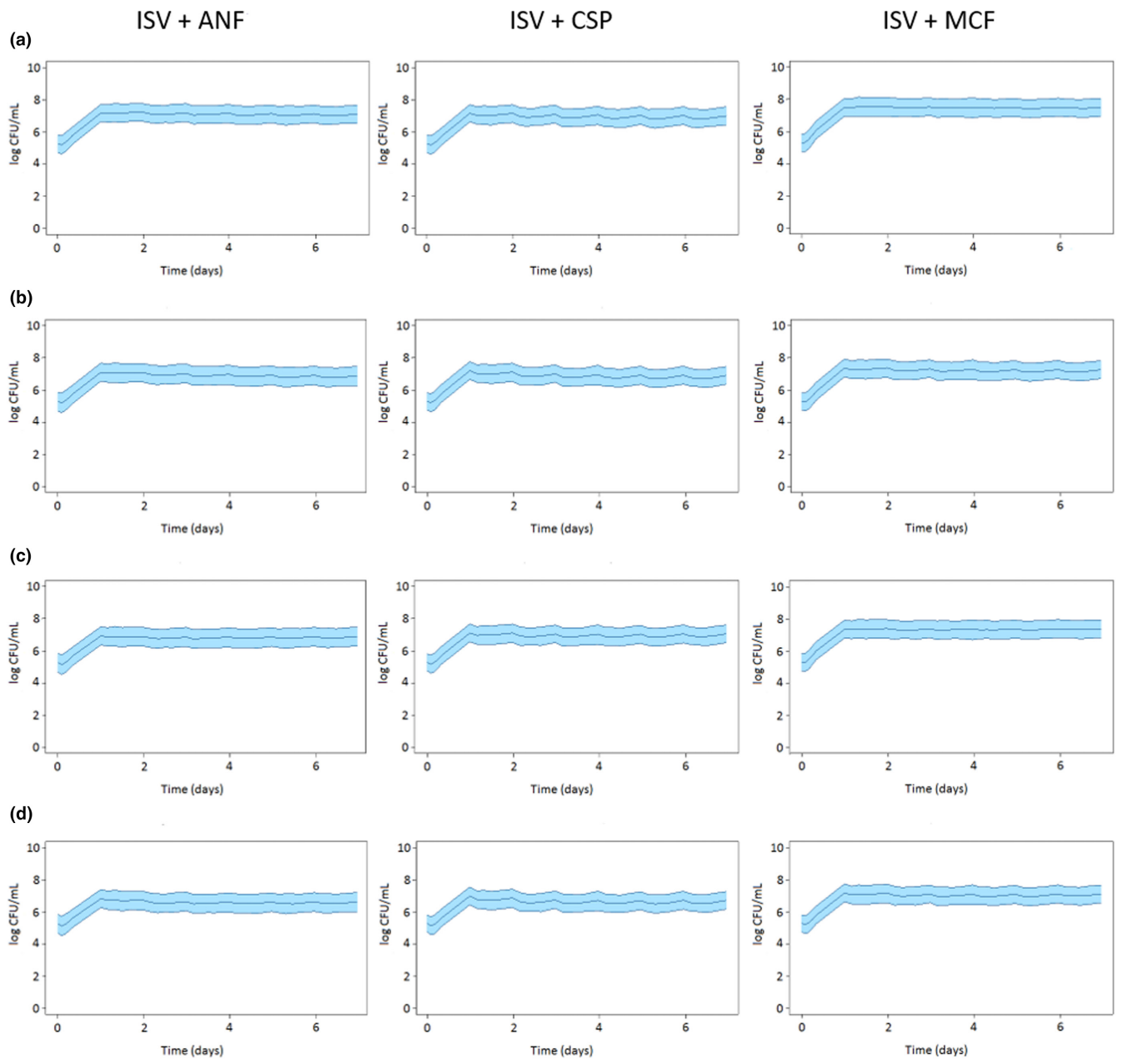

FIGURE 4 Effect on the fungal burden of different dosing-regimens of isavuconazole + anidulafungin (ISV + ANF), isavuconazole + caspofungin (ISV + CSP) and isavuconazole + micafungin (ISV + MCF). (a) standard dosing of both isavuconazole and echinocandins, (b) standard dosing of isavuconazole + alternative dosing of echinocandins, (c) alternative dosing of isavuconazole + standard dosing of echinocandin, (d) alternative dosing of both isavuconazole and echinocandins. The mean (solid line) and 95% prediction interval (colored space) are represented.

for echinocandins. These MIC thresholds for high-dosing combination therapy were similar to the susceptibility-breakpoints for anidulafungin and micafungin established by EUCAST for *C. albicans* and *C. glabrata*.[37] In a study by Bader et al.,[34] a PK/PD evaluation of the target attainment was conducted for echinocandins against *C. glabrata* infections. To sum up, this study suggested that regardless of dosing increases of anidulafungin and micafungin, these two drugs are unlikely to provide therapeutic exposures against isolates with elevated MICs, whereas caspofungin does. The results of the present study are in line with this conclusion, as the combination with caspofungin was the most active one against *C. auris*. Although there are no susceptibility-breakpoints for isavuconazole yet, the threshold of 0.03 mg/L in combination therapy also resembles the conclusions of Wu et al.[21] driven by Monte Carlo simulations and the probability of target attainment with standard monotherapy treatment against invasive candidiasis. Overall, this highlights the importance of bridging the in vitro

TABLE 2 Summary of different dosing regimens for the combination of isavuconazole with anidulafungin or caspofungin and MIC scenarios for which fungal growth was inhibited.

MIC_{ISV} (mg/L)	MIC_{CANDIN} (mg/L)	Minimum dose requirements ISV+ echinocandins			
		ISV	ANF	ISV	CSP
0.015	0.015	Licensed	Licensed	Licensed	Licensed
0.03	0.015	Licensed	Alternative	Licensed	Alternative
		Alternative	Licensed	Alternative	Licensed
0.015	0.03	Licensed	Alternative	Licensed	Licensed
		Alternative	Licensed		
0.03	0.03	Alternative	Alternative	Licensed	Alternative
				Alternative	Licensed
0.015	0.06	Alternative	Alternative	Licensed	Alternative
				Alternative	Licensed
0.03	0.06	x	x	Alternative	Alternative

Note: Licensed regimens: ISV, 200 mg/8 h first 48 h, and 200 mg/day after; ANF, 200 mg loading dose and 100 mg/day; CSP, 70 mg on day 1 followed by 50 mg/day from day 2. Alternative regimens: ISV, 400 mg/8 h first 48 h, and 200 mg/day after; ANF, 200 mg loading dose and 200 mg/day; CSP, 100 mg/day.
Abbreviations: ANF, anidulafungin; CSP, caspofungin; ISV. isavuconazole; MIC_{ISV}, minimum inhibitory concentration of isavuconazole; MIC_{CANDIN}, minimum inhibitory concentration of the echinocandin.

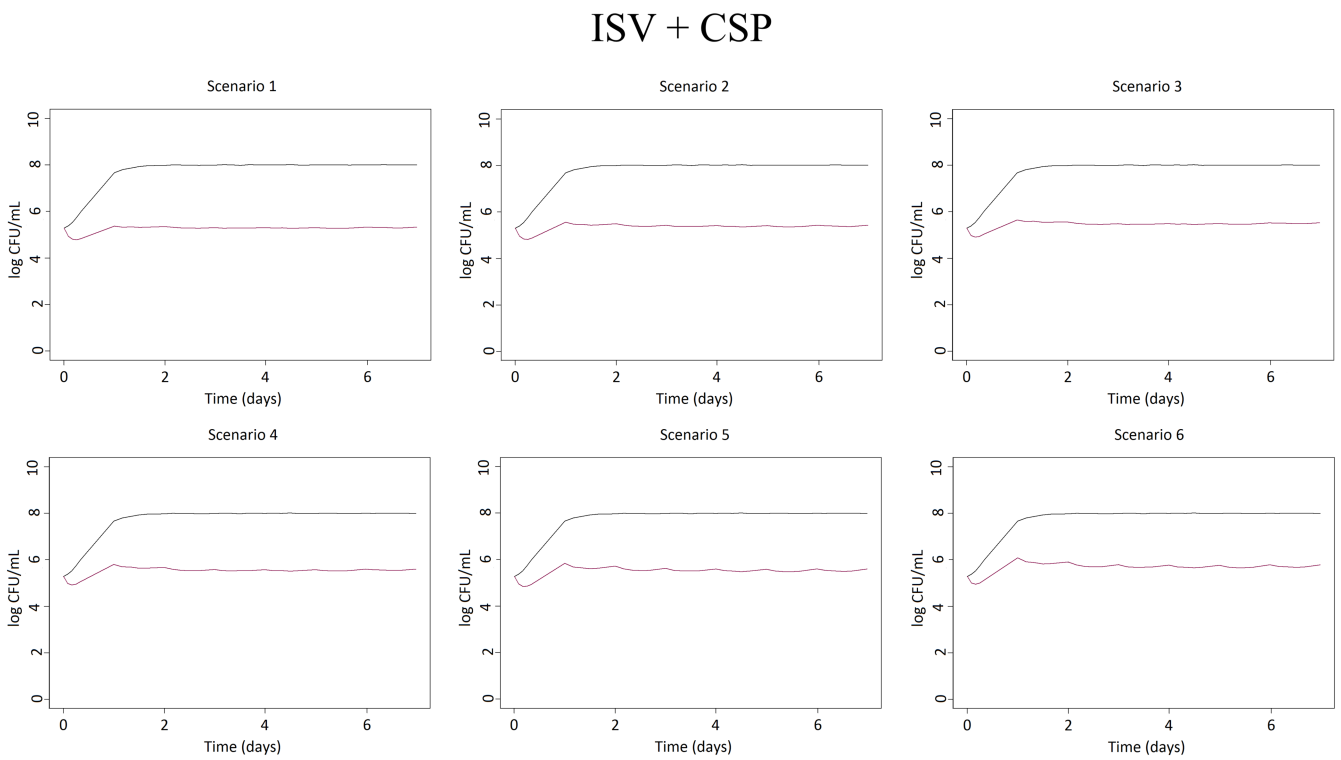

FIGURE 5 Effect on the fungal burden of the combination of proposed alternative dosages of isavuconazole and caspofungin (ISV + CSP). Black line represents growth control (no treatment) and the magenta line represents the mean outcome of the treatment arm. Scenario 1: MIC of 0.015 mg/L for ISV and 0015 mg/L for CSP. Scenario 2: MIC of 0.03 mg/L for ISV and 0.015 mg/L for CSP. Scenario 3: MIC of 0.015 mg/L for ISV and 0.03 mg/L for CSP. Scenario 4: MIC of 0.03 mg/L for ISV and 0.03 mg/L for CSP. Scenario 5: MIC of 0.015 mg/L for ISV and 0.06 mg/L of CSP. Scenario 6: MIC of 0.03 mg/L for ISV and 0.06 mg/L for CSP. MIC, minimum inhibitory concentration.

results to an in vivo scenario, as conclusions regarding therapeutic use may change drastically. However, bridging from in vitro to in vivo is also challenging because there are some important considerations that need to be addressed, as discussed below. These limitations can be discussed under three headings, which are: drug protein-binding, tissue-distribution of the antifungal drugs, and the *C. auris* isolates studied.

Because echinocandins are highly bound to plasma proteins, when the total plasma concentrations after standard treatments are corrected by the theoretical unbound fraction, the calculated free drug concentrations are usually below the MIC, and simulation outcomes may point erroneously to therapeutic failures.[38] In vitro experiments have shown that serum indeed affects the activity of antifungal drugs compared to protein-free mediums, but the increase in MIC or minimal fungicidal concentrations in those works were not as high as predicted by the unbound-fraction.[39–42] Ishikawa et al.[41] investigated and compared the activity of micafungin in RPMI medium and in serum from patients and evidenced an antifungal activity in serum much higher than the anticipated by a free fraction of 0.02%. They suggested that the binding of micafungin might be weak and reversible, and that in the presence of *Candida*, it releases from the protein and binds to the fungal target. Elefanti et al.[40] also used a similar reasoning for anidulafungin but suggested that the shift from bound to unbound drug might be not so prominent in vivo, because the total volume of drug distribution is much bigger than the volume of infection, which is the opposite of the in vitro setting. Interestingly, Kovács et al.[43] recently found that echinocandins were more active in serum supplemented RPMI than in standard RPMI against *C. auris*. In this case, the authors stated that high concentrations of echinocandin might stimulate chitin synthesis as a compensatory mechanism; lower free drug concentrations in serum-supplemented media would not trigger that biosynthesis, thus paradoxically leading to a higher killing activity. In summary, the efficacy outcomes in our study are correlated with the free fraction of each drug, as caspofungin, the echinocandin with the lowest protein binding, was predicted to be the most active, whereas the combination with micafungin, a drug with a protein binding as high as 99.9%, would be the least active. Nevertheless, taking into account the former works, the approximation of correcting the total plasmatic level by the free fraction for these highly bounded drugs may be too simplistic. It is very likely that the combination of isavuconazole plus micafungin might have a greater in vivo activity than the predicted one.

Another complex in vivo factor not accounted for in simulations is the tissue distribution of antifungal drugs. Echinocandins are widely and rapidly distributed into body compartments affected by invasive candidiasis, achieving higher concentrations in tissue than in plasma.[25] Louie et al.[44] observed in a murine model of systemic candidiasis that whereas the concentration of caspofungin in plasma was below the MIC, the concentration in kidney tissue was much higher and, thus, better explained the antifungal activity. Anidulafungin also remains longer in these tissues than in plasma, as proved in animal models.[45,46] Gumbo et al.[45] stated that the tissue concentrations of anidulafungin in rats are in the order of the estimated EC_{50}, and, therefore, more closely related to the observed effect in clinical practice. Conversely, micafungin tissue concentrations are more similar to the ones in plasma, but the antifungal effect is persistent even when tissue concentrations are below the MIC.[47] Regarding isavuconazole, studies in both animals and humans have shown that this drug is well-distributed into tissue and concentrations are high enough to exert an effect.[48,49]

Finally, another limitation of the present study is that all the studied *C. auris* isolates belonged to the same clade, closely related to the South African one.[18] In future studies, it would be interesting to include isolates classified in the different clades of *C. auris*, at it has been suggested that the degree of antifungal activity is highly clade-specific.[13,43,50] In this sense, the incorporation of the isolates from the different clades in the modeling and simulation approach could yield valuable results of clinical applicability.

In conclusion, the developed PK/PD model was able to characterize properly the antifungal activity of isavuconazole in combination with echinocandins against *C. auris*. By linking the in vitro based PK/PD model to population PK clinical information, combined antifungal therapy was translated into a clinical setting. Model-based simulations predicted that the combinations of isavuconazole with anidulafungin or caspofungin would be effective for MICs up to 0.03 and 0.06 mg/L, respectively, whereas the combination with micafungin would lead to treatment failure. Further studies are needed to better understand the interaction between drugs and fungal targets in vivo and, thus, to strengthen simulation-based decision making.

AUTHOR CONTRIBUTIONS

U.C., N.J., E.E., G.Q., and V.V. wrote the manuscript. U.C., N.J., E.E., and G.Q. designed the research. U.C., N.J., and E.E. performed the research. U.C., V.V., S.S., and N.J. analyzed the data.

ACKNOWLEDGMENTS

The authors wish to thank doctors Javier Pemán and Alba Ruiz Gaitán (Hospital Universitario y Politécnico La Fe, Valencia, Spain) for kindly providing clinical isolates.

FUNDING INFORMATION

This research was funded by Consejería de Educación, Universidades e Investigación of Gobierno Vasco-Eusko Jaurlaritza, GIC15/78 IT-990-16, FIS PI17/01538, and MCINN PID2020-117983RB-I00. U.C. was funded by a PhD grant from the University of the Basque Country, PIF 17/266.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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How to cite this article: Caballero U, Eraso E, Quindós G, Vozmediano V, Schmidt S, Jauregizar N. PK/PD modeling and simulation of the in vitro activity of the combinations of isavuconazole with echinocandins against *Candida auris*. *CPT Pharmacometrics Syst Pharmacol*. 2023;12:770-782. doi:10.1002/psp4.12949